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Proposal for Research

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A NEW APPROACH TO THE DETECTION AND DEVELOPMENT OF ANTIBIOTIC MATERIALS

(embodies SL Memoprieis)
see p. 5

Prepared for:

Bristol Laboratories, Inc.
Syracuse 1, New York

Approved:

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Introduction

The search for new antibiotics, stimulated by the great value of those now available, continues at a high rate of effort.

The usual process of developing a new antibiotic from a biological source begins with the detection of a promising antibiotic-producing organism, followed by the isolation, structural determination, then extensive chemical modification and testing of the potential antibiotic. It is a slow, laborious process because of the fact that only a few organisms excrete potential antibiotic materials in usable quantities.

A shorter route to new antibiotic materials -- the subject of this proposal -- might be found by making use of the many potentially valuable precursor materials that may exist within the cell. Such materials, which escape most of the present search patterns for usable microbiological products, could be modified to an almost infinite variety of forms by simple physical and chemical methods. The various modified products could then be screened for antibiotic or other medicinal functions. Any substance which exhibited promise would, of course, be subjected to closer scrutiny, and its active component could then be isolated.

Objectives

The primary objective of this proposed project is to investigate the feasibility of developing new antibiotics and/or other types of medicinal compounds by screening chemically-treated cellular debris from certain organisms.

The secondary objective, to be undertaken to a degree of effort specified by the project advisory committee (see below), is to attempt to elucidate something of the chemical nature of promising materials developed during the first part of the project.

Method of Approach

The project will be divided into two phases, corresponding to the primary and secondary objectives.

Phase I - Obtaining, Treating, Screening Cellular Debris

A suitable microorganism such as Escherichia Coli will be grown in order to obtain the necessary cellular tissue. Since this is primarily a feasibility study, only amounts of the selected organism necessary for testing will be grown. The research will approximately follow the outline presented below:

1. The crude cellular residue will be obtained by simple centrifugation.

2. The cellular residue will be subjected to one-dimensional paper chromatography and bioautographed on a Staphlococcus organism culture plate or another suitable organism culture plate.

3. The cellular residue will be subjected to various hydrolytic or other disruptive processes (physical and chemical) and then screened as in Step 2, above.

4. The cellular residue will be treated with various chemical agents (see below) and then screened as in Step 2.

5. Any mixtures exhibiting antibiotic properties will be used in Phase II of the project. Other mixtures will be discarded.

The chemical treatment of the cellular residues will be carried out in 100 to 500 ml volumes and will involve such methods as follow:

1. Reaction with fluorodiazomethane. This would lead to many derivatives of acidic components.

2. Reaction with ethylene oxide. This would lead to alkylation of many active sites such as amino groups, hydroxyl groups and other functions.

3. Partial sulfonation to obtain sulfonated derivatives of potential activity.

4. Partial chlorination to obtain mixtures of many halogenated biological products.

5. Reaction with thioacetic acid.

6. Reaction with ethyleneimine.

7. Reaction with thionyl chloride.

8. Reaction with liquid ammonia under pressure.

9. Reaction with any other suitable reagents as may come to mind.

The screening process might be readily conducted by obtaining bioautographic evaluations and by running rough, one-dimensional paper chromatograms of the treated cellular materials with controls. A product will be considered biologically active if a reaction mixture of 10-20 percent purity, containing 10-20 micrograms of solids/ml, inhibits the growth of Staphlococcus aureus. Any promixing mixtures could be rechecked with two-dimensional paper chromatography and bioautographic scanning before any isolation program was undertaken.

Phase II - Studies of Promising Antibiotics

This phase of the work will, of course, depend on results obtained during Phase I and will be planned in detail (in consultation with the project's advisors) as the work progresses.

Generally, it is expected that attempts would be made to isolate the active components of any mixtures showing promise in Phase I. Such methods as large-scale paper chromatography, column chromatography, counter current distribution electrophoresis, or other methods that seem feasible or expedient will be considered. Attempts will also probably be made to isolate and concentrate sufficient quantities of the active fraction to aid in identification and evaluation studies.

Qualifications of Stanford Research Institute

The Institute is a 10-minute drive from Stanford University and about an hour's drive from the University of California at Berkeley, thus facilitating communication with consultants working in those universities. Also available to project personnel are a wide range of experts in many disciplines here at the Institute. Such a "multi-disciplined" approach is invaluable in a project of this nature.

Personnel likely to work directly on this project are:

Leon Goodman, Senior Organic Chemist., Ph.D., University of California at Los Angeles. He has done research involving halogen addition to olefins containing neighboring functional groups at the University of California, research on synthesis and properties of aryl sulfonate esters at the University of Southern California, research on synthesis

of organic high explosives at Los Alamos, and research on synthesis of polar silane molecules. He is assistant project leader of the cancer chemotherapy synthesis program being carried out by Stanford Research Institute.

Lloyd K. Moss, Biochemist, Ph.D., Stanford University, 1957. He has had extensive experience in all phases of chromatography. During the past two years he has supervised the chromatographic work of the cancer chemotherapy synthetic program at the Institute. This has involved paper and column chromatography of many compounds, including amino acid analogs, carbohydrates and derivatives, purine bases, nucleosides and nucleotides, steroids, and fraudulent vitamins. His experience in enzymology includes isolation, assay, and chemical characterization.

R. M. Silverstein, Senior Organic Chemist, Ph.D., New York University, 1949. Dr. Silverstein's fields of experience include synthesis of heterocyclics, sulfur-containing compounds, metal-organics, silicon monomers containing functional groups, quaternary ammonium compounds and synthetic estrogens; mechanism studies of tautomeric and geometric isomeric systems, of oxidative gelation of silicone fluid and of silane-olefin reactions; development of plasticizers, defoamers, high temperature antioxidants, detergents, and chemicals from fats and oils; analysis of insecticides, and food flavors.

Janet E. Morris, Microbiologist, M.A., Stanford University, is experienced in medical technology and microbiology.

D. Karen Anderson, Organic Chemist, M.S., Northwestern University, is experienced in reactions of halogens with vinyl ethers.

Personnel available for consultation are:

B. R. Baker, Program Director, Bio-organic Chemistry, Ph.D., University of Illinois. Dr. Baker has carried out research on synthesis and isolation of vitamins, and on synthesis of organic materials of medicinal interest: tuberculosis, cancer, trypanosomiasis, malaria, schistosomiasis, worm diseases, vitamins, antibiotics, plant materials, pharmacodynamic compounds, nucleosides, nucleotides, purines, and other heterocycles, etc. He is the director of the cancer chemotherapy synthesis program being carried out by Stanford Research Institute and is a member of the Chemistry Panel of Cancer Chemotherapy National Service Center, National Cancer Institute. He has over 90 publications and 100 patents.

Joseph Greenberg, Program Director, Microbiology, Ph.D., Harvard University. Before coming to the Institute, Dr. Greenberg was head of the Section on Chemotherapy, the National Institutes of Allergy and Infectious Diseases of the U. S. Public Health Service. He has had considerable experience in parasitology research, with particular emphasis on malaria and amebiasis. Currently he is using microbiological techniques in the chemotherapeutic study of cancer.

Chozo Mitoma, Neurochemist, Ph.D., University of California, 1951. Studies on the biochemistry of the central nervous system often include references to Dr. Mitoma's notable contributions. They cover such areas as the formation of 5-hydroxytryptophan from tryptophan, bipsyhthesis of γ-guanidinobutyric acid from γ-aminobutyric acid, the demonstration of monoamine oxidase activity with tetrazolium salts, and the effect of ortho- and meta-tryamines on the central nervous system and its implications in phenylketonuria. He has been a unit head in the Laboratory of Clinical Biochemistry together with Dr. Sidney Udenfriend for the past seven years. Dr. Mitoma will be joining the SRI staff in the near future.

Reports and Meetings

A Steering Committee, composed of Institute and Bristol Laboratories personnel, will direct the work of the project and establish the level of effort to be undertaken in Phase II. It is understood that Dr. Joshua Lederberg of Stanford University will be a member of the Steering Committee and will also act in an advisory capacity throughout the entire project.

It is proposed to have a meeting of the Steering Committee at or near the close of Phase I in order to determine the direction of Phase II work. Periodic reports on the progress of the work will be submitted at the request of the client. A final report will describe all research results and conclusions obtained by the research team.

Estimated Time and Charges

It is estimated that nine months will be required to complete this project and report its results. The Institute could begin work on the project immediately following approval of this proposal.

The estimated maximum charges for this project are $24,750. This amount will not be exceeded without prior approval of the client.

Stanford Research Institute is a not-for-profit, nonendowed organization. In view of this status, the Institute requests each research client

to make an advance deposit to cover working capital requirements for the project being undertaken. The Institute's invoicing procedure is described in its standard research agreement. The usual advance deposit on a project of this size is $5500. If the research client wishes to eliminate payment against periodic invoices, the advance deposit amount may be increased up to 100 percent of authorized funds. Upon completion of a project, any unexpended project funds on hand are returned to the client.

Acceptance Date

This proposal will remain in effect until May 31, 1959. If consideration of the proposal requires a longer period, the Institute will be glad to consider a request for an extension in time.

Respectfully submitted,

Bruce Graham

Bruce Graham, Chairman
Department of Biological Sciences

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